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# THIN-LAYER CHROMATOGRAPHY: TLC

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**Thin-layer chromatography** (TLC) is used for identifying compounds and determining their purity. The most common adsorbent used is **silica gel**, but **Alumina** is gaining popularity, and with good reason. Compounds should separate the same on an alumina plate as on an alumina column, and column chromatography using alumina is still very popular. It is very easy to run test separations on **TLC plates** rather than on **chromatographic columns**.

Nonetheless, both of these adsorbents are powdered and require a **solid support**. **Microscope slides** are extremely convenient. To keep the powder from just falling off the slides, manufacturers add a *gypsum binder* (plaster). Adsorbents with the binder usually have a “**G**” stuck on the name or say “For thin-layer use” on the container.

Sometimes a fluorescent powder is put into the adsorbent to help with **visualization** later. The powder usually glows a bright green when you expose it to **254-nm wavelength ultraviolet (UV) light**. You can probably figure out that if a container of silica gel is labeled **Silica Gel G-254**, you’ve got a TLC adsorbent with all the bells and whistles.

Briefly, you mix the adsorbent with water, spread the mix on the microscope slide in a *thin layer*, let it dry, and then activate the coating by heating the coated slide on a hot plate. Next you **spot** or place your unknown compound on the plate, let an **eluent** run through the adsorbent (**development**), and finally examine the plate (**visualization**).

## PREPARATION OF TLC PLATES

1. Clean and dry several microscope slides.
2. In an Erlenmeyer flask, weigh out some adsorbent and add water.
  - a. For silica gel use a 1:2 ratio of gel to water. About 2.5 g gel and 5 g water will do for a start.
  - b. For alumina, use a 1:1 ratio of alumina to water. About 2.5 g alumina and 2.5 g water will be a good start.
3. Stopper the flask and shake it until all the powder is wet. This material must be used quickly because a gypsum (plaster) binder is present.
4. Spread the mix by using a *medicine dropper*. *Do not use disposable pipets!* The disposable pipets have *extremely* narrow openings at the end, and they clog up easily. There exists a “dipping method” for preparing TLC slides, but since the usual solvents, methanol and

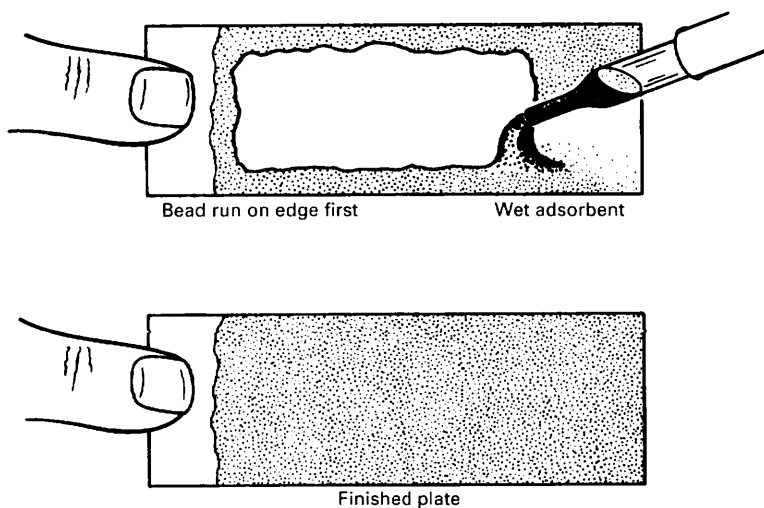
chloroform (**Caution! Toxic!**) do not activate the binder, the powder falls off the plate. Because the layers formed by this process are very thin, they are very fragile.

5. Run a bead of mix around the outside of the slide, and then fill the remaining clear space. Leave  $\frac{1}{4}$  inch of the slide blank on one end, so you can hold onto the slide. Immediately tap the slide from the bottom to smooth the mix out (Fig. 116). Repeat this procedure with as many slides as you can. If the mix sets up and becomes unmanageable, add a little water and shake well.
6. Let the slides sit until the gloss of water on the surface has gone. Then place the slides on a hot plate until they dry.

(**Caution!** If the hot plate is too hot, the water will quickly turn to steam and blow the adsorbent off the slides.)

## THE PLATE SPOTTER

1. The **spotter** is the apparatus used to put the solutions you want to analyze on the plate. You use it to make a spot of sample on the plate.
2. Put the center of a **melting point capillary** into a small, blue Bunsen burner flame. Hold it there until the tube softens and starts to sag. Do not rotate the tube, ever.

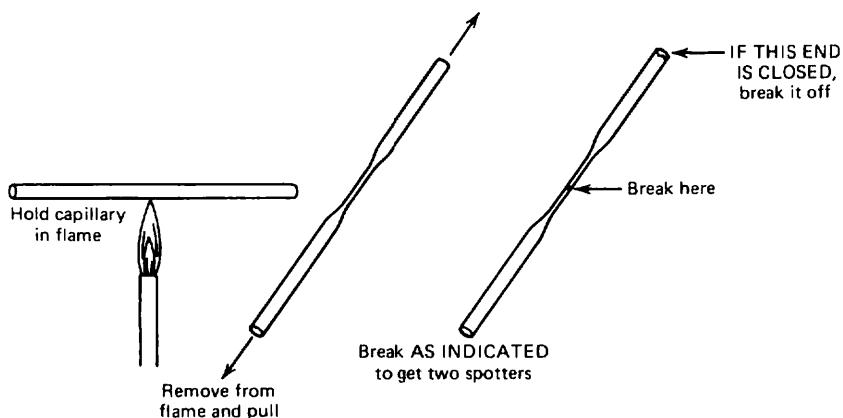


**Fig. 116** Spreading adsorbent on a TLC plate.

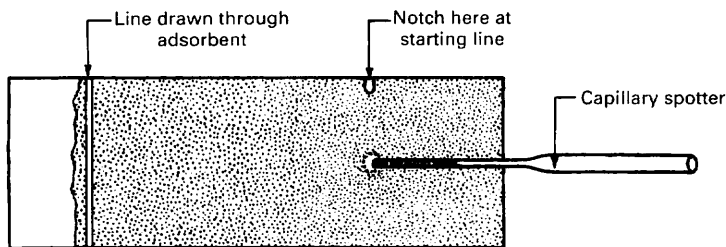
3. *Quickly* remove the capillary from the flame, and pull both ends (Fig. 117). If you leave the capillary in the flame too long, you get an obscene-looking mess.
4. Break the capillary at the places shown in Fig. 117 to get **two** **spotters** that look roughly alike. (If you've used capillaries with *both ends open already*, then you don't have a closed end to break off.)
5. Make up 20 of these, or more. You'll need them.
6. Because TLC is so sensitive, spotters tend to "remember" old samples if you reuse them. *Don't put different samples in the same spotter.*

## SPOTTING THE PLATES

1. Dissolve a small portion (1–3 mg) of the substance you want to chromatograph in *any* solvent that dissolves it *and* evaporates rapidly. Dichloromethane or diethyl ether often works best.
2. Put the thin end of the capillary spotter into the solution. The solution should rise up into the capillary.
3. Touch the capillary to the plate *briefly*! The compound will run out and form a small spot. Try to keep the spot as small as possible—not larger than  $\frac{1}{4}$  inch in diameter. Blow gently on the spot to evaporate the solvent. Touch the capillary to the *same place*. Let this new spot grow to be *almost the same size as the one already there*. Remove the capillary and gently blow away the solvent. This will build up a concentration of the compound.



**Fig. 117** Making capillary spotters from melting point tubes.

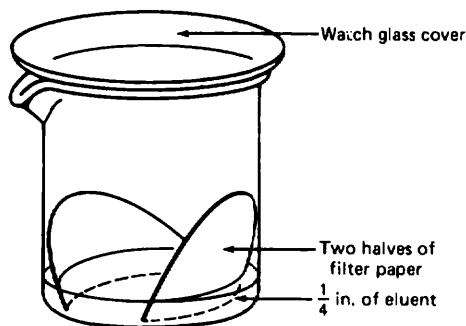


**Fig. 118** Putting a spot of compound on a TLC plate.

4. Take a sharp object (an old pen point, capillary tube, spatula edge, etc.) and *draw a straight line through the adsorbent*, as close to the clear glass end as possible (Fig. 118). Make sure that it runs all the way across the end of the slide and goes right down to the glass. This will keep the solvent from running up to the ragged edge of the adsorbent. It will travel only as far as the smooth line you have drawn. Measurements will be taken from this line.
5. Now make a small notch in the plate at the level of the spots to mark their starting position. You'll need this later for measurements.

## DEVELOPING A PLATE

1. Take a 150 ml beaker, line the sides of it with filter paper, and cover with a watch glass (Fig. 119). Yes, you can use other containers. I've used beakers because all chemistry stockrooms have beakers, you know what beakers look like, and it's a handy single word for the more

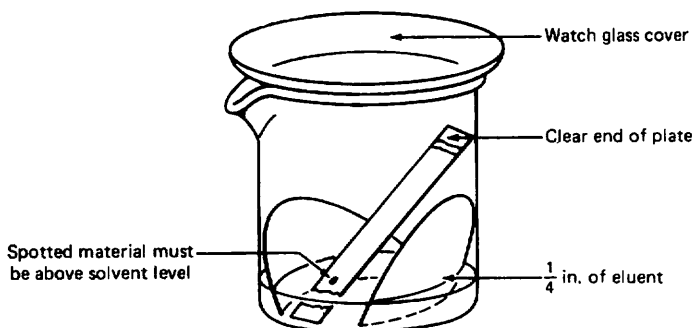


**Fig. 119** The secret identity of a 150 ml beaker as a TLC slide development chamber is exposed.

cumbersome “thin-layer development chamber used at your institution.” So if you do use jelly jars, drinking glasses, wide-mouth screw-cap bottles or whatever, just substitute that description for beaker in this discussion, OK?

2. Choose a solvent to **develop** the plate. You let this solvent (eluent) pass through the adsorbent by capillary action. Nonpolar eluents (solvents) will force nonpolar compounds to the top of the plate, whereas polar eluents will force *both polar and nonpolar materials up the plate*. There is only one way to choose eluents. Educated guesswork. Use the chart of eluents presented in Chapter 25.
3. Pour some of the eluent (solvent) into the beaker, and tilt the beaker so that the solvent wets the filter paper. Put no more than  $\frac{1}{4}$  inch of eluent in the bottom of the beaker! This saturates the air in the beaker with eluent (solvent) and stops the evaporation of eluent from the plate.
4. Place the slide into the developing chamber as shown (Fig. 120). *Don't let the solvent in the beaker touch the spot on the plate*, or the spot will dissolve away into the solvent! If this happens, you'll need a new plate, and you'll have to clean the developing chamber as well.
5. Cover the beaker with a watch glass. The solvent (eluent) will travel up the plate. The filter paper keeps the air in the beaker saturated with solvent so that it doesn't evaporate from the plate. When the solvent reaches the line, *immediately* remove the plate. Drain the solvent from it, and blow gently on the plate until *all* the solvent is gone. If not, there will be some trouble visualizing the spots.

*Don't breathe the fumes of the eluents!* Make sure you have adequate ventilation. Work in a hood if possible.



**Fig. 120** Prepared, spotted TLC plate in a prepared developing chamber.

## VISUALIZATION

Unless the compound is colored, the plate will be blank and you won't be able to see anything, so *you must visualize the plate*.

1. ***Destructive visualization.*** Spray the plate with sulfuric acid, then bake in an oven at 110°C for 15–20 minutes. Any spots of compound will be charred blots, utterly destroyed. *All* spots of compound will be shown.
2. ***Semidestructive visualization.*** Set up a developing tank (150 ml beaker) but leave out the filter paper and any solvent. Just a beaker with a cover. Add a few crystals of **iodine**. Iodine vapors will be absorbed onto most spots of compound, coloring them. Removing the plate from the chamber causes the iodine to evaporate from the plate, and *the spots will slowly disappear. Not all spots may be visible.* So if there's nothing there, that doesn't mean nothing's there. The iodine might have reacted with some spots, changing their composition. Hence the name **semidestructive visualization**.
3. ***Nondestructive visualization.***
  - a. ***Long-wave UV (Hazard!)*** Most TLC adsorbents contain a fluorescent powder that glows bright green when you put them under long-wave UV light. There are two ways to see the spots:
    - (1) The background glows green, and the spots are dark,
    - (2) The background glows green, and the spots glow some other color.*The presence of excess eluent may cause whole sections of the plate to remain dark. Let all the eluent evaporate from the plate.*
  - b. ***Short-wave UV (Hazard!)*** The plates stay dark. *Only the compounds may glow.* This is usually at 180 nm.

Both of the UV tests can be done in a **UV light box** in a matter of seconds. Since most compounds are unchanged by exposure to UV, the test is considered **nondestructive**. Not everything will show up, but the procedure is good enough for most compounds. When using the light box, *always turn it off when you leave it*. If you don't, not only does the UV filter burn out, but also your instructor becomes displeased.

Since neither the UV nor the iodine test is permanent, it helps to have a record of what you've seen. You must *draw an accurate picture of the*



*plate in your notebook.* Using a sharp-pointed object (pen point, capillary tube, etc.), you can trace the outline of the spots on the plate while they are under the UV light (**Caution!** Wear gloves!) or before the iodine fades from the plate.

## INTERPRETATION

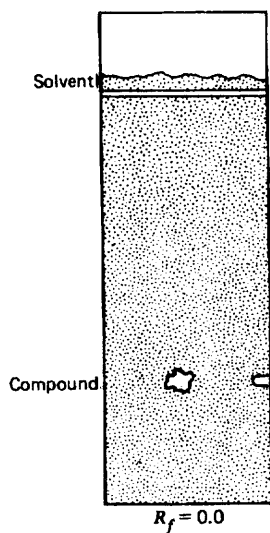
After visualization, there will be a spot or spots on the plate. Here is what you do when you look at them.

1. Measure the distance *from that solvent line* drawn across the plate to *where the spot started*.
2. Measure the distance from *where the spot stopped* to where the spot began. Measure to the *center of the spot* rather than to one edge. If you have more than one spot, get a distance for each. If the spots have funny shapes, do your best.
3. Divide the *distance the solvent moved* into the *distance the spot(s) moved*. The resulting ratio is called the  **$R_f$  value**. *Mathematically*, the ratio for any spot should be between 0.0 and 1.0, or you goofed. *Practically*, spots with  $R_f$  values greater than about 0.8 and less than about 0.2 are hard to interpret. They could be single spots or multiple spots all bunched up and hiding behind one another.
4. Check out the  **$R_f$  value**—it may be helpful. In identical circumstances, this value would always be the *same* for a single compound, all the time. If this were true, you could identify unknowns by running a plate and looking up the  $R_f$  value. Unfortunately, the technique is not that good, but you can use it *with some judgment* and a *reference compound* to identify unknowns (see “Multiple Spotting,” following).

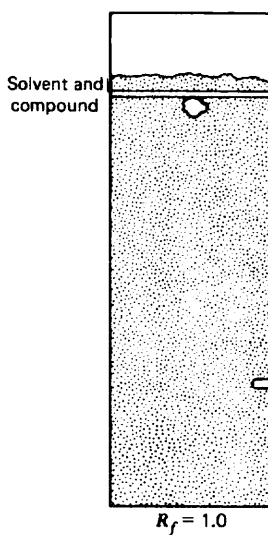
Figures 121 to 123 provide some illustrations. Look at Fig. 121: if you had a mixture of compounds, you could never tell. This  $R_f$  value gives no information. Run this compound again. Run a new plate. *Never redevelop an old plate!*

Use a more polar solvent!

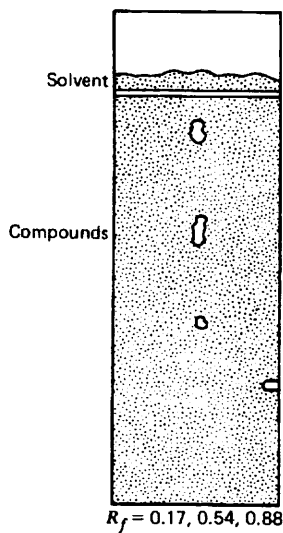
No information in Fig. 122 either. You couldn't see a mixture if it were here. Run a new plate. *Never redevelop an old plate!*



**Fig. 121** Development with a nonpolar solvent and no usable results.



**Fig. 122** Development with a very polar solvent and no usable results.



**Fig. 123** Development with just the right solvent is a success.

Use a less polar solvent!

If the spot moves somewhere between the two limits (shown in Fig. 123) and *remains a single spot*, the compound is pure. If *more than one spot shows*, the compound is impure and it is a **mixture**. Whether the compound should be purified is a matter of judgment.

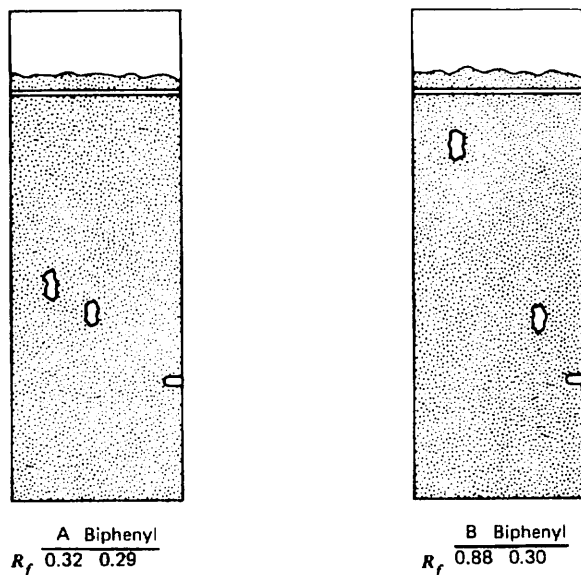
## MULTIPLE SPOTTING

You can run more than one spot, either to save time or to make comparisons. You can even *identify unknowns*.

Let's say that there are two unknowns, A and B. Say one of them can be biphenyl (a colorless compound that smells like moth balls). You spot two plates: One with A and biphenyl, side by side, and the other, B and biphenyl, side by side. After you develop both plates, you have the results shown in Fig. 124.

Apparently, A is biphenyl.

Note that the  $R_f$  values are not perfect. This is an imperfect world, so don't panic over a slight difference.



**Fig. 124** Side-by-side comparison of an unknown and a leading brand known.

And now we have a method that can quickly determine

1. Whether a compound is a mixture.
2. The identity of a compound *if a standard is available*.

There are **pre-prepared plates** that have an active coating on a thin plastic sheet, also with or without the fluorescent indicator. You can cut these to any size (they are about 8 inches by 8 inches) with a pair of scissors. *Don't touch the active surface with your fingers—handle them only by the edge.* The layers on the plate are much thinner than those you would make by spreading adsorbent on a microscope slide, so you have to use smaller amounts of your compounds in order not to overload the adsorbent.

## CO-SPOTTING

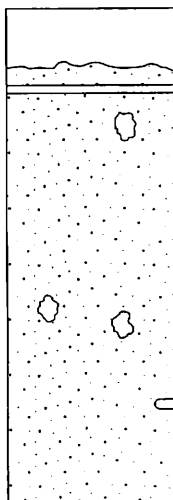
You can nail down identification with **co-spotting**, but it is a bit tricky. This time, spot one plate with your unknown, A, in two places. Now let the spots dry entirely. I mean entirely! Now we'll spot biphenyl *right on top of one of the spots of A*. (If you didn't let the earlier spot dry entirely, the biphenyl spot would bleed and you'd have a *very* large—and useless—spot of mixed compounds.) Do the same for B on another plate. Now run these plates.

The  $R_f$  values might be a bit different, but there's only *one spot* in the biphenyl over the A column. No separation. They are the same. If you see two (or more) spots come from co-spotting, the substances, like B, are not the same (Fig. 125).

## OTHER TLC PROBLEMS

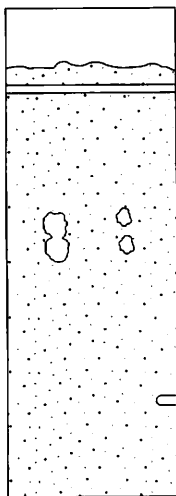
What you've seen is what you get if everything works OK. You can:

1. Make the spots too concentrated. Here spots smear out, and you don't see any separation. Dilute the spotting solution or don't put so much on (Fig. 126).
2. Put the spots too close together. Here you could get the spots bleeding into each other, and you might not be able to tell which spot came from which origin (Fig. 127).



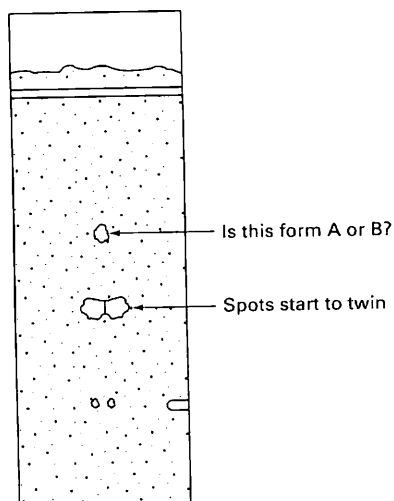
|       | A<br>over<br>biphenyl | B<br>over<br>biphenyl |
|-------|-----------------------|-----------------------|
| $R_f$ | 0.32                  | 0.88<br>and<br>0.29   |

**Fig. 125** Co-spotting biphenyl over A and B.



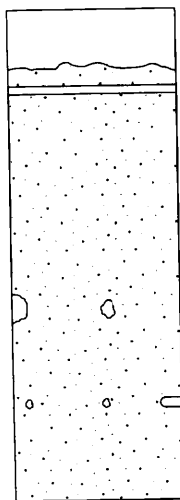
Concentrated mixture      Diluted mixture

**Fig. 126** Effect of concentration on separation.



**Fig.127** Effect of spots being too close together.

3. Spot too close to the edge. Here you get inaccurate  $R_f$  values, bleeding, and other problems. The spots are not surrounded as much by adsorbent and solvent, so unequal forces are at work here (Fig. 128).



**Fig.128** Effect of spot too close to edge.

## PREPARATIVE TLC

When you use an **analytical technique** (like TLC) and you expect to **isolate compounds**, it's often called a **preparative (prep) technique**. So TLC becomes **Prep TLC**. You use the same methods, only on a larger scale.

Instead of a microscope slide, you usually use a 12 X 12 inch glass plate and coat it with a thick layer of adsorbent (0.5-2.0 mm). Years ago, I used a small paintbrush to put a line (a streak rather than a spot) across the plate near the bottom. Now you can get special plate streakers that give a finer line and less spreading. You put the plate in a large developing chamber and develop and visualize the plate as usual.

The thin line separates and spreads into bands of compounds, much like a tiny spot separates and spreads on the analytical TLC plates. Rather than just look at the bands, though, you *scrape the adsorbent holding the different flasks*, blast your compounds off of the adsorbents with appropriate solvents, filter off the adsorbent, and finally evaporate the solvents and actually recover the separate compounds.